Formalin

CAS ID: 50-00-0

Chemical formula: C_2H_20

Synonyms / Trade names: Formacide-B™, Formalin-F, Paracite-S®

Chemical composition

Formalin (100 %) is a generic term that describes a solution of 37% formaldehyde gas dissolved in water. Approximately 10% to 15% methanol is added to inhibit the formation of paraformaldehyde, a precipitate considered toxic to fish. Because the formaldehyde solution will polymerize, methyl alcohol is used as a stabilizer. The FDA has approved three formulations of formalin for use in aquaculture Formalin F, Formacide –B™ and Paracite –S®; all contain 37% by weight formaldehyde gas in water. Formacide –B is 37% formaldehyde, 6 to 14% methanol and 49-57% water and inert ingredients. Parasite-S is another trade name for formalin. All of these formulations have the same CAS number and the toxicity data does not specify which trade names were tested only formalin with methanol, and formaldehyde. Formaldehyde is an extremely reactive compound that interacts with protein, RNA and DNA in vitro. [1]

Formalin is prepared in various formulations for use in aquaculture which vary only in the amount of methanol composition. The formulations listed above have been approved for use by the FDA on fish intended for human consumption (Francis-Floyd 1996). Apparently formalin does not persist in fish tissue at concentrations of concern, as there is no legal withdrawal time from when the chemical is administered and when the fish can be slaughtered for consumption.

Hatchery Use

The Parasite-S formulation is administered in a bath treatment to control for external protozoa (*Chilodonella spp., Costia spp., Epistylis spp., Ichthyophthirius spp., Scyphidia spp. and Trichodina spp*.), and the monogenetic trematode parasites *(Cleidodiscus spp., Dactylogyrus spp., and Gyrodactylus spp*.) on all finfish. It is also used for the control of fungi of the family *Saprolegniaceae* on all finfish eggs (Western Chemical Label, no date).

Measures of Exposure

Formalin is administered to salmon and trout as a bath treatment. The standard dosage recommended in the INAD #9013 Protocol to prevent or control fungus on fish and eggs is 15 - 2000 µL/L (ppm) in a static-bath or flow-through treatment at. Eggs are treated daily or every other day until hatch. Fish are treated every other day to weekly[2] for 30 to 60 minutes, and then transferred to clean water (Francis-Floyd 1996) . The formalin concentration is water temperature dependent and 50^{o} F is the cutoff for the two treatment concentrations. Salmon and trout are treated up to 170 µL/L at water temperatures above 50^{o} F and 250 µL/L at temperatures below 50^{o} F. All other finfish are treated up to 250 µL/L regardless of temperature. Treatment is not recommended to exceed 1.0 hour (FDA 1995).

[1] http://cmr.asm.org/content/12/1/147.full
[2] http://www.fws.gov/fisheries/aadap/summaryHistoryFormalin.htm accessed 8/12/2014

Because formalin removes oxygen from the water its use in closed systems in discouraged. Formalin removes dissolved oxygen (DO) at a 5:1 ratio, for every 5 mg/L (ppm) formalin applied, 1 mg/L of DO is removed from the water. Formalin is also toxic to algae, reducing photosynthesis through increasing respiration and decomposition (Francis-Floyd 1996). The manufacturers label directs the user to dilute the formalin treated water by 10 and 100 times when discharging from ponds, raceways and hatch houses, respectively (Western Chemical, Inc., no date).

Environmental Fate of Formalin

If released into water, formaldehyde is not expected to adsorb to suspended solids and sediment based upon the estimated K_{oc}. Formaldehyde readily biodegrades under both aerobic and anaerobic conditions in the environment assuming that the concentration is not toxic to microbes. In a die-away (biodegradation) test using water from a stagnant lake, degradation was complete in 30 and 40 hr under aerobic and anaerobic conditions, respectively. Volatilization from water surfaces is not expected to be an important fate process based upon the Henry's Law constant (3.4 x 10-7 atm-m^3/mole). It is not expected to bioaccumulate in aquatic organisms, formalin is metabolized and transformed by them through various metabolic pathways[3].

An estimated bioconcentration factor of 3 suggests the potential for bioconcentration in aquatic organisms is low. Microbial degradation is the main breakdown mechanism because of the lack of hydrolyzable functional groups[4].

According to FDA (1995) formalin:

- contains 37 to 40% formaldehyde with 10 to 15% added methanol.
- has molecular weight of 30.03.
- is miscible with water, alcohol and acetone.
- has a pH of the 2.8 to 4.0.

  Common chemical reactions consist of:

  1) $H_2C = O + H_2O \rightarrow H_2C - (OH)_2\ (Formalin\ or\ Formaldehyde\ hydrate)$

  2) $2H_2C = O + O_2\ \rightarrow 2HCOOH \rightarrow (Formic\ Acid)$

  3) $HCOOH + O_2\ \rightarrow CO_2 + H_2O\ (Carbon\ Dioxide\ and\ Water)$

Equation #1 depicts the formation of formalin through the mixture of formaldehyde gas and water. Equation #2 presents the oxidation of formalin or formaldehyde to formic acid which is broken down by microorganisms to carbon dioxide and water as shown in equation #3. The ultimate mineralization of formaldehyde to carbon dioxide and water indicates that environmental impacts will be minimal. The

[3] http://www.inchem.org/documents/ehc/ehc/ehc89.htm#SectionNumber:1.3

[4] http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=712&loc=ec_rcs#x351

primary concern is the potential for impacts to sensitive aquatic organisms upon exposure to elevated surface water or expected environmental concentrations (EEC).

Expected Environmental Concentrations

Formalin is administered at concentrations ranging from 170 to 250 µL/L to fish, and up to 2000 µL/L to eggs. The therapeutic concentration selected depends on the temperature and density of organisms in the treatment structure, as both along with the formalin influence the level dissolved oxygen (DO) (Table F-1).

Table F-1. Dosage (ppm) for the control of external parasites on fish and fish eggs

Aquatic Species	**Administer in tanks and Raceways for up to 1 hour (µl/L)**	**Administer in Earthen Ponds indefinitely (µl/L)**
Salmon and Trout Up to 50 ^{o}F Below 50 ^{o}F	 up to 170 up to 250	 15 to 25 15 to 25
All other finfish	up to 250	15 to 25
Eggs of all finfish except Acipenseriformes	1000 to 2000 for 15 minutes	
Eggs of Acipenseriformes	up to 1500 for 15 minutes	

The potential for exposure to non-target species is through the discharge of formalin treated raceways or hatchery house water. The rate of discharge depends upon the size of the treatment pond, raceway or proportion of the egg stacks treated.

Formalin is applied to raceways after the water supply is turned off, the appropriate amount of formalin is added along with aeration to facilitate mixing. The treatment is administered for up to one hour, after which raceway water is replaced by clean aerated water.

Formalin is applied to ponds in a dilute formulation using a pump, sprayer, boat bailer or other mechanism to assure mixing. In most cases a single treatment is efficacious, however if retreatment is necessary it occurs after 5 to 10 days (FDA 1995) . The use of formalin in ponds is likely to result in a lower receiving water concentrations as formalin is applied at initial concentrations of 15-25 ppm, the half-life is 36 hrs, and flow through would be minimal until the formalin is dispelled. If the parasite is *Ichthyophthirius sp*. Formalin is administered for two-day intervals until control of the pathogen is achieved (FDA 1995).

FDA suggests that the receiving water concentrations of effluent from treatment tanks or raceways after dilution should not exceed 1 ppm (FDA, 1995). In the finding of no significant impact for Parasite-S®, FDA requires a 10-fold dilution of finfish treatment water and a 100-fold dilution of finfish egg treatment water, which should lead to a discharge concentration of no more than 25 µl/L[5]. Because this is the

[5] http://water.epa.gov/scitech/wastetech/guide/aquaculture/upload/2005_09_01_guide_aquaculture_EEBA_EEBA-Chapter-7.pdf

level of dilution is required by the label we assume that the EEC at the end of the discharge pipe will not exceed this concentration.

In order to compare the formalin EEC to toxicity data for formalin and formaldehyde presented in different units, we must first convert ppm reported as µl/L to mg/L using the density of formalin (1.086 g/ml), then we convert formalin to formaldehyde:

Formalin EEC: 25 µl/L x (1.089 g/ml) = 27 mg/L formalin

Formaldehyde EEC: 27 mg/L Formalin * 0.37 (% formaldehyde) = 10 mg/L formaldehyde

Measures of Effect

The toxicity of formalin is primarily attributed to the formaldehye as the toxicity of methanol to fish is low. During their derivation of the acute and chronic water quality criteria Hohreiter and Rigg (2001) conducted an ECOTOX search for methanol, as many of the studies they considered were testing formalin, which contains 10% to 15% methanol. According to these studies the 96 hr LC_{50} of methanol alone was greater than 10,000 mg/L. Hohreiter and Rigg (2001) used the EPA methodology (Stephan et al. 1985) to derive the criteria. They considered data for 12 species of fish species, 3 species of amphibians and 11 species of invertebrates; they note that chronic data were limited. When studies presented concentrations on a volume by volume (µl/L) basis, the authors converted to a weight-basis (mg/L) by multiplying the concentrations by the density of formalin (1.089 g/ml), this density is also used along with the percent formaldehyde in the formulation to convert formalin to formaldehyde (as shown above). Using these data Hohreiter and Rigg (2001) calculated a final acute value (FAV) of 9.15 mg/L and a final ACR using available chronic toxicity data and the EPA-recommended conservative default assumptions to account for missing data. The authors calculated the following aquatic life criteria for formaldehyde:

FAV 9.15 * ½ = 4.58 mg/L

ACR = 5.69

FCV = 1.61 mg/L

Acute Toxicity of Formalin and Formaldehyde - Salmonid

As previously stated, concentrations up to 250 µl/L are used to treat external parasites on salmon and trout. It stands to reason that the short-term therapeutic dose would not result in mortality of the fish being treated, which is supported by the data presented in Tables F-2 and F-3. Formalin toxicity is positively correlated with exposure time, rainbow trout LC_{50}'s range from 1,407 to 2,400 ppm and decrease to 100 ppm over 96 hrs. Not unexpectedly, there are cases where the therapeutic concentration has resulted in latent mortality (2 to 4 hr after exposure) of some individuals (4 to 6 % of the test population, but this is within the acceptable control mortality according to standard toxicity testing.

The toxicity of formalin varies depending on the species and size of the fish. Of the salmonids tested the majority of the data reported results for rainbow trout (Table F-2), which have been reported to be the most sensitive species (Smith and Piper 1972). Small fish are more tolerant to formalin exposure than large fish.

Taylor and Glenn (2008) exposed two different size classes of fish to formalin. Their 'small' group of fish had a target body weight of 2 grams, while their 'large' group of fish had a target body weight of 10 grams. Fish were exposed to formalin bath of various concentrations for one hour, then placed in clean water for an additional 120 hours to identify any residual mortality response from exposure to the formalin bath. Concentrations of formalin used in the one hour bath were 108.9, 217.8, 326.7, 435.6, 544.5, 653.4, 762.3 and 871.2 mg/L. The 96 hour LC_{50} values for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) were calculated from a logistic response function. The equation used was that given below.

$$Y_i = \frac{e^{(\beta_0 + \beta_1 x_j)}}{1 + e^{(\beta_0 + \beta_1 x_j)}}$$

Where: Y_i = mortality probability (= 0.50)

β_0 = logistic regression intercept

β_1 = logistic regression slope

x_j = chemical concentration (mg/L)

Calculated 96 hour LC_{50} values are given in Table F-2 Taylor and Glenn (2008) did not report confidence intervals around their LC_{50} values.

Table F-2. Juvenile rainbow trout (steelhead) mortality data and confidence intervals for varying static test durations.

Exposure Duration (hr)	Formalin LC_{50} (95% CI) (µl/L)	Adjusted LC_{50}/EC_{50} mg/L Formaldehyde	Water Temp. (^oC)	Reference
1	2310 (1959-2724	930.8 (789.3 – 1097.6)	12	(Bills et al. 1977)
	1407 (mg/L) (NR)	520.6	13	(Taylor and Glenn 2008)
3	1230 (957 – 1581	495.6 (385 – 637)	12	(Bills et al. 1977)
6	>400	>161.2	12	(Howe et al. 1995)
	655 (580-740	264 (233.7 – 298.2)	12	(Bills et al. 1977)
24	220 (198-245)	88.6 (79.8 – 98.7)	12	(Howe et al. 1995)
	300 (237 – 380	120.9 (95.5 – 153.1)	12	(Bills et al. 1977)
96	117 (100 – 136)	47.1 (40.3 – 54.8)	12	(Marking et al. 1984)
	121 (101 -144)	48.7 (40.7 – 58.0)	12	(Howe et al. 1995)
	122(102 – 146)	49.2 (41.1 – 58.8)	12	(Marking et al. 1984)
	118 (98 – 140)	47.5 (40.3 – 56.4)	12	(Bills et al. 1977)

Data are compiled from literature searches conducted in 2008 and 2014.
Assumed that the Howe et al. 1984 was reporting formalin with 37% formaldehyde not reported in the paper
NR: Not reported

Table F-3. 96-hour static acute toxicity data for tests with other Salmonid species.

Species	Effect	Formalin Concentration mg/L	Adjusted LC50 mg/L Formaldehyde	Reference
Chinook	LD 2	304	112.5	(Taylor and Glenn 2008)
	LD50	563	208.3	(Taylor and Glenn 2008)
Lake Trout	LC50	109 (84.9 – 139.4)	40.2 (31.3 – 51.4)	(Bills et al. 1977)
Coho	LD2	653	241.6	(Taylor and Glenn 2008)
	LD50	840 mg/L	310.0	(Taylor and Glenn 2008)
Atlantic Salmon	LD50	188 (162.3-218.9)	64.0 (55.1 – 74.4)	(Bills et al. 1977)

Units converted from µl/L to mg/L.
NA: Not applicable
NR: Not reported

Interspecies Correlation Models

Empirical data including growth and smoltification and lethality were available for both steelhead (rainbow trout) and Chinook, respectively (Table F-2 and F-3). EPA used Rainbow trout, lake trout and Atlantic salmon as surrogates in the ICE model for predicting LC$_{50}$s for the other ESA-listed salmonid species (Table F-4). The output of the ICE model runs are presented in Appendix F-X with the results used to estimate chronic no observed effect concentrations (NOEC's) for salmonid species highlighted in green. The remaining ICE models, with poorer predictive ability and which were not selected as the source are also presented in this appendix.

Chronic data needed to calculate acute to chronic ratios (ACRs) and generate chronic NOECs (NOECs) was very limited. Hohreiter and Rigg (2001) calculated an ACR of 5.69 in their derivation of water quality criteria for formaldehyde based on having data for at least one species in eight different families. As recommended by EPA the default value of 20 was used for fish because the fish data didn't meet the minimum requirements set out in Stephan et al (1985) for development of the final ACR. The Stephan et al. (1985) guideline don't specify how ACRs for the various taxa should be combined to calculate the final ACR, therefore Hohreiter and Rigg (2001) calculated a geometric mean to come up with the Final ACR of 5.69. Raimondo et al (2007) developed the national ACR (8.3) by taking the median value for all data, chronic test type, general taxa, ambient media, chronic test end point, and chemical mode of action (MOA)/class groupings for the 456 same species pairs. The national ACR appeared to be driven by narcosis point, and chemical mode of action (MOA)/class groupings Raimondo et al. (2007).

The chronic NOEC values are presented in Table F-4. As described in the problem formulation, the lower 95% confidence interval (CI) of the predicted toxicity estimation (in this case the 96 hr LC$_{50}$), was used along with the National ACRs to calculate the chronic NOECs in this BE.

Table F-4. Chronic no observed effect concentrations (NOEC) for formalin and T&E salmonids

Species	**Formalin (mg/L)**	**Formaldehyde mg/L**	**Source of chronic NOEC**
Bull trout	10.4	4.3	ICE model – genus level
Chinook salmon	181.9	67.3	Empirical data (NOEC for growth and seawater challenge)
Sockeye salmon	12.4	5.0	ICE model – genus level
Steelhead	19.9	7.3	Empirical data
Chum Salmon	12.4	5.0	ICE model – genus level
Coho Salmon	12.4	5.0	ICE model – genus level

EPA ran the ICE model at the at the genus level for all ESA-listed salmonids except Chinook salmon and steelhead. We calculated the species mean acute value (SMAV) using the geometric mean of the toxicity data for each surrogate species where possible (Appendix X). We then used the lowest 95% CI of the geometric means of the 96 hr LC_{50}'s as the input parameter for each species in the WEB-ICE model. The model output for rainbow trout had the best fit with both rainbow trout and sockeye salmon. Since steelhead and rainbow trout are the same species, we used the empirical (LC_{50}) data directly to predict toxicity to steelhead. We adjusted the output of the ICE models with the ACR developed by Hohreiter and Rigg (2001) of 5.69. A NOEC (70 µL/L) was listed in the EPA Office of Pesticide Program Ecotoxicity Database citing the Bills et al. (1977) paper, but we were unable to find any mention of this NOEC in the paper itself, therefore we defaulted to using the national ACR to predict the Chronic NOEC (Table F-4).

Smith et al. (1987) measured gill ATPase (enzyme activity correlated with seawater tolerance), growth and survival in a seawater challenge test using Chinook to evaluate a particularly sensitive life stage of anadromous salmonids. Fish were exposed to formalin for 1 hour every two weeks for six weeks and then subjected to the seawater challenge test. The authors found no significant difference in survival and growth in between the formalin treated (167µL/L) fish and the control group. The authors conclude that formalin at a concentration of 167 µL/L (182 mg/L) is safe for smolts and pre-smolts.; this value was used as the chronic NOEC for Chinook salmon.

Use of the ICE model and the ACR resulted in lower NOEC values than reliance on empirical data. Bull trout, chum and sockeye salmon all have lower NOEC values than steelhead which, according to the literature is more sensitive to formalin than other salmonids.

Sublethal Toxicity of Formalin - Salmonids

The use of therapeutic chemicals often results sublethal effects (or side-effects in the case of human pharmaceuticals) that impact the homeostatic functions of the organism undergoing treatment. The presumption is that the condition being treated would be more deleterious if left unchecked than the sublethal effects from the use of the drug.

Most of the formalin studies tested the standard therapeutic doses (Table F-1); these are the concentrations to which fish are routinely exposed, so there is interest in determining whether sublethal effects are occurring, and if treatment modifications would lessen any of these effects (Wedemeyer and Yasutake 1974; Bills et al. 1977; Smith 1984; Smith et al. 1987). Exposure periods often mimic standard dosing periods of 30 min to 1 hr; other exposure periods were extended up to 6 hours (Wedemeyer 1971). Some studies included a single short-term exposure period coupled with longer term monitoring (Smith and Piper 1972; Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Smith et al. 1987; Taylor and Glenn 2008). Nieminen et al. (1983) tested multiple exposures separated by 24 hr periods simulating repeated treatment to control parasites.

In order to attribute a measurable effect to a sublethal endpoint it is necessary to understand how the endpoint effects the organism in a way that reduces its fitness or survival. The interpretation of a meaningful biological consequences of immunological, histopathological and hematological responses to formalin exposure is necessary in order to predict a measurable effect in the organism. Unless explicitly tested, it's difficult to predict the reduction in an organism's fitness in a quantitatively meaningful way, particularly when the affect elicits a short-term response and has a low ecological relevance (Adams et al. 1989).

Adams et al. (1989) recommended the use of bioindicators which include a suite of identified stress responses representing various levels of biological significance to evaluate the sublethal effects on fish from exposure to environmental contaminants. The authors identified levels of biological response along gradients of response time and toxicological and ecological significance. Homeostatic indices including detoxification enzymes, immunological and histopathological measures are considered short-term responses at lower ecological significance, while condition, reproductive competence and population and community indices are long-term responses with greater ecological relevance.

Various blood parameters and liver histopathology indicative of fish health and chemotherapeutic stress are routinely evaluated as secondary effects fish physiology and on metabolism following formalin treatment (Wedemeyer 1971; Smith and Piper 1972; Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Nieminen et al. 1983; Smith et al. 1987) (Table 4). These measures conform primarily to short-term responses of low ecological relevance, with gill and liver pathology representing conditions of greater ecological significance (Adams et al. 1989).

Table F-5. Documented no effect and sublethal effects from the use of formalin on Salmonid species.

Species	Effect	Concentration	Endpoint	Reference
Chinook	NOEC	167 (µl/L)/182 mg/L	Growth or survival during seawater challenge/smoltification progress	(Smith et al. 1987)
	NOEC	200 (µl/L)/217 mg/L	Hematological parameters; gill pathology	(Wedemeyer and Yasutake 1974)
Rainbow Trout	NOEC	200 (µl/L)/ 217 mg/L (up to 1 hr exposure)	Hematological parameters; gill pathology	(Wedemeyer and Yaksutake 1974)
	EC	250 and 1250 (µl/L)[1] / 272 and 1361 mg/L (2X/30 min/24hrs)	Chemotherapeutic stress	Nieminen et al 1983
	EC	200 (µl/L)/217 mg/L (1 hr bath treatments)	Hematological and hepatic responses indicative of regulatory stress	(Williams and Wootten 1981)
Atlantic Salmon	EC	250 and 1250 (mg/L) 272 and 1361 mg/L (2X/30 min/24hrs)	Chemotherapeutic stress	(Nieminen et al. 1983)
Coho	NOEC	200 (mg/L)	Hematological parameters	(Wedemeyer 1971)

[1]: Assumed the ppb (reported in the paper) is in µl/L

Exposure to formalin at therapeutic concentrations results is a stress response in salmonids that is more pronounced in rainbow trout. The stress response in measured through changes in blood chemistry that influence the homeostatic balance in fish (Wedemeyer 1971; Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Nieminen et al. 1983). A second notable marker of exposure commonly measured includes gill and liver pathology (Wedemeyer 1971; Smith and Piper 1972; Wedemeyer and Yasutake 1974).

Various authors have demonstrated recovery in salmon (Coho and Chinook) and steelhead after 24 hrs from 1 hr exposures to therapeutic concentrations of formalin; they concluded that the stress of a 1 hr treatment was not great enough to result in changes in the blood resulting in respiratory alkalosis (increased respiration increasing the pH of blood) or liver pathology; repeated exposures resulted in significant sublethal effects (Wedemeyer and Yasutake 1974; Williams and Wootten 1981).

Niemenin et al. (1983) exposed Atlantic salmon and rainbow trout for two and four 30 min treatments to 272 and 1,361 mg/L formalin, respectively (in excess of the therapeutic concentration). They found no significant difference blood glucose levels (an indicator of stress) between Atlantic salmon and rainbow trout and control fish after a single 30 min exposure to 272 mg/L formalin. Response continued to be insignificant in the salmon upon a second 30 min exposure and only a slight significance in rainbow trout after this second treatment. Mortality occurred when salmon and trout were exposed four times for 30 mins to 272 and 1,361 mg/L at 24hr intervals (Nieminen et al. 1983). Formalin is not permitted for use at these concentrations and exposure frequencies in standard aquaculture practices.

Rainbow trout experience greater effects on the gill epithelial layer when exposed to formalin, this may be the noted difference in response between this species and other salmonids tested (Wedemeyer 1971; Nieminen et al. 1983).

Exposure to formalin at therapeutic concentrations triggers a stress response in treated fish only after repeated short-term (30 min) exposures at elevated concentrations (272 and 1,361 mg/L). The frequency of exposure and allowable recovery period influence the magnitude of the sublethal response. After one hour exposure gill pathology ranges from limited biological significance (217 mg/L) to severe (272) mg/L, with no effect on growth, survival or osmoregulation at the lowest therapeutic concentration tested (181 mg/L). Fish generally recover from homeostatic stress responses after a 24 hr period, however, repeated exposures at or exposures in excess of treatment levels can result in damage to gills. It is unlikely that the concentration of formalin in receiving waters (EEC) would exceed 181mg/L to 217 mg/L given that these are the therapeutic dosages and dilution of the treatment water will occur prior to and even more so after discharge. Additionally the FDA requires that the formalin EEC does not exceed 1 mg/L after dilution in the receiving water. Finally, it's important to note that the EECs will be compared to the chronic NOEC values for T&E species to determine the potential for adverse effect, these NOEC values (Table F-4) are lower (by at least half) than the sublethal effect levels reported in the literature.

Both lethal and sublethal endpoints were reported from numerous literature sources. Lethality (LC_x) was measured more frequently than sublethal effects, however biochemical and histological parameters and growth were reported as well (Table F-5). Although we discuss homeostatic measures representing sublethal endpoints, EPA did not rely on these modes of action (e.g. change in blood chemistry) if they could not be attributed to a measurable effect in the ESA-listed, non-target, or surrogate species. Instead we relied on endpoints representative of long-term ecologically relevant responses in fish including survival, growth and reproduction; where available these data were used to estimate chronic no-adverse-effect concentrations (NOEC) using the ICE model as described in the problem formulation section of this BE.

Risk Characterization

Risks to ESA-listed Fish Species from formalin and Formaldehyde

Risks to ESA-Listed fish species for which toxic concentrations of formalin and formaldehyde can be identified from the literature are calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the estimated environmental concentration is divided by the chronic NOEC for each ESA-listed species to calculate a hazard quotient. Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this BE, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of a T&E species. Note that acceptable chronic NOEC values vary between species. Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to ESA-listed species.

Hazard quotients for the nine six ESA-listed salmonid species for which toxicity data is available or could be estimated are presented in Tables F-8 and F-9. Hazard quotients were calculated using the EEC resulting from the therapeutic concentration (272 mg/L) and required (100x) dilution stipulated by FDA which results in the largest EEC (27 mg/L) to which ESA Listed species could be exposed.

Table F-8. Hazard quotients (HQ) for ESA-listed species exposed to the expected environmental concentrations (EEC) of formalin discharged by aquaculture facilities

Species	EEC (mg/L)	Chronic NOEC (mg/L)	Hazard quotient
Bull trout	27	10.4	2.6
Chinook salmon	27	181.9	0.15
Sockeye salmon	27	12.4	2.2
Steelhead	27	13.6	1.4
Chum salmon	27	12.4	2.2
Coho salmon	27	12.4	2.2

Table F-9. Hazard quotients (HQ) for ESA-listed species exposed to the expected environmental concentrations (EEC) of formaldehyde discharged by aquaculture facilities

Species	EEC (mg/L)	Chronic NOEC (mg/L)	Hazard quotient range
Bull trout	10	4.3	2.5
Chinook salmon	10	67.3	0.15
Sockeye salmon	10	5.0	2.0
Steelhead	10	7.4	1.3
Chum salmon	10	5.0	2.0
Coho salmon	10	5.0	2.0

All hazard quotients in Tables F-8 and F-9 are higher than 1.0, indicative of potential ecological risk to the species under the standard dilution stipulated on the label (25 µl/L). Note that the EEC values do not take into account the actual duration of exposure of species present in the receiving water, which is likely to be less than a constant 96 hours. As presented earlier the toxicity of formalin increases with exposure duration, which is no doubt one reason why the fish are treated with the drug for up to one hour and the frequency of treatment is repeated every other day.

Lethality is not expected to occur at the EEC even at a 96hr exposure duration therefore, effects to the species would be sublethal in nature. Because formalin is used as a therapeutic agent in aquaculture and hatchery operations where the goal is to produce and maintain healthy fish, conditions indicative of chemotherapeutic stress are routinely evaluated as secondary effects on fish physiology and metabolism following formalin treatment. EPA has discussed these and identified levels of formalin that are associated with these stress responses (Table F-5). Because formalin is administered for a short periods of time the evaluations of secondary toxicity have also been designed for short (30 min to 1 hour) exposures. As presented in Table F-5 the concentrations resulting in these secondary effects range from 200 to 1400 mg/L, well in excess to the 10 mg/L to 27 mg/L EECs for formaldehyde and formalin, respectively. However, the HQs calculated for the estimated NOECs exceed 1.0 at the EEC for most species for 96 hour tests; the true effect level lies somewhere in between at a particular exposure duration, and both are significant contributors to the uncertainty of the assessment. Another significant source of uncertainty in the lack of chronic data.

Uncertainty Analysis of Formalin Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this formalin evaluation.

Variation of expected environmental concentrations in aquaculture discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of formalin use is short term and irregular (parasite and fungi control). Because of this use pattern, prediction of exposure duration in receiving waters is confounded and would be expected to be on the order of hours and not days. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC_{50}, EC_{50}, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this BE for species where no empirical toxicity data exists for a chemical-species pair.

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